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Stem Cell Labeling, Tracking, and Delivery in Cardiovascular Disease

Introduction:

The poor regenerative capacity of the heart has lead to the exploration of exogeneous cellular therapies. The potential of these therapies to-date has been determined using models that require serially animal sacrifice for histological analysis. Recently, magnetic labeling of cells, with clinically approved contrast agents in an off-label use, followed by transplantation and transfusion has enabled non-invasive tracking of cellular distribution with MRI in animal models. In addition, these MR cellular labeling techniques have now been explored in non-cardiac applications in patient studies in Europe.(1) Thus, using preclinical animal models the optimal dosing and timing can be determined by non-invasive cardiac MR serial imaging. Moreover, these techniques are well poised to make the transition to human clinical trials for both efficacy studies and tailoring of individual patient treatment.

Cellular Labeling Strategies:

Most methods for MR cellular labeling are based on techniques developed with monoclonal antibodies linked to radionuclides for radionuclide imaging. However, the low background signal of radionuclide imaging allows for the detection of extremely low numbers of cells. Thus, MR methods require a 10-100 fold larger amplification for similar detection of cellular targets. However, the advantage of labeling with MR contrast agents is two-fold: 1.) the cells are not exposed to radioactive species, which are often cytotoxic when internalized, and 2.) the lack of a half-life of MR contrast agents such that the cells can be imaged for 2-6 months post-transplantation if cellular proliferation is small.

MR contrast agents can be divided into two major classes of agents: the paramagnetic and superparamagnetic iron oxide contrast agents. Gadolinium chelates, which form the most widely used clinically approved MR contrast agents, are in the paramagnetic class of agents. The adoption of gadolinium-based MR contrast agents for non-cellular imaging is largely due to the ability of these agents to decrease the T1 relaxation time at low doses. This decrease in T1 results in an increase signal intensity (i.e., hyperintense signal) of tissues exposed to the contrast agent using T_1-weighted imaging sequences. However, due to the high toxicity of free gadolinium, chelation of gadolinium is paramount. Concerns about dechelation of the gadolinium compounds in applications for genetic and cellular labeling have largely limited the development of these agents for these purposes. More problematic is the reduced ability of paramagnetic compounds when internalized to affect extracellular water, and, hence, the amplification ability of the contrast agent is greatly reduced.

The superparamagnetic iron oxide (SPIO) particles were developed shortly after the gadolinium-based contrast agents.(2,3) The large magnetic moments, which are more than 3 orders of magnitude greater than paramagnetic-based contrast agents, of SPIO particles cause a greater effect on T_2 relaxation and a smaller effect on T_1 relaxation. Thus, on T_2^*-weighted images, SPIO particles appear hypointense and create a much larger signal change or contrast per unit of metal particle than paramagnetic contrast agents. Thus, small quantities of SPIO particles can be used for cellular labeling yet with a much larger amplification effect than paramagnetic compounds. This is important

because less agent must be internalized to create image contrast thereby limiting cellular toxicity. Moreover, should the SPIO be degraded, the free iron that is released does not appreciably expand the native iron pool and, thus, can be degraded along normal iron recycling pathways. Most commercially available forms of SPIOs and ultrasmall SPIOs (USPIOS) have coatings to prevent particle aggregation. One of the most common U/SPIO coatings is dextran, which is a convenient surface for binding ligands and other functional groups for labeling.

Gadolinium-based labeling techniques:

Modo and colleagues have developed a gadolinium-based compound linked to dextran and a fluorescent dye, rhodamine, that can be taken up by stem cells *in vitro* for intracellular labeling (4). These exogenously labeled stem cells have been implanted in a rat stroke model, and the migration has been tracked *in vivo* by MRI and validated by detection of the fluorescent label by histology (5). Using a calcium phosphate transfection technique, Rudelius *et al.* performed intracellular labeling of neuronal and embryonic stem cells with Gd-DTPA (6). Using this labeling technique Daldrup-Link *et al.* were able to achieve intracellular labeling of hematopoietic progenitor cells that resulted in significant lengthening of R_1 such that detection of 500,000 cells or greater should be possible (7). However, as stated previously, the internalization of these Gd particles will greatly reduce the potential contrast enhancement imparted by these agents. Thus, due to amplification problems and toxicity concerns, iron oxide compounds remain the preferred agents for cellular labeling.

One exception is a targeted nanoparticle containing a liquid perfluorocarbon core with chelated gadolinium (Gd) complexes incorporated into the outer surface group developed by the group of Wickline, Lanza, and co-workers .(8,9) They have overcome the amplification problem with gadolinium-based agents by two means: 1.) numerous Gd molecules can be bound to the surface of the nanoparticle and 2.) antibody receptor complexes on the nanoparticle are used for targeting so that the particle is not internalized which would reduce T1 relaxivity. Thus, changes in T1 relaxation can be achieved *in vivo*. In addition, therapeutics incorporated into the surface of the nanoparticle for drug delivery.(10,11) By modifying the antibody receptor complex, targeting of this agent has been performed to fibrin (i.e., thrombus) for cardiovascular applications.(8,12,13) To decrease the potential toxicity of the gadolinium chelate, several paramagnetic formulations have been explored, which have also increased the relaxivity of the agent resulting in enhanced signal enhancement after injection.(12) The most exciting cardiovascular application is the targeting to markers of angiogenesis (i.e., $\alpha_v\beta_3$ integrins) using this agent.(13,14) The fluorine signal of the nanoparticle can also be detected with MR spectroscopy providing an independent method for validating the bright Gd signal detected in proton imaging. In addition, the introduction of higher field strength magnets for clinical imaging may overcome the signal-to-noise issues associated with ^{19}F spectroscopy and imaging.

U/SPIO labeling techniques:

The initial methods for cellular MR labeling with dextran-coated iron oxides were based on radionuclide monoclonal antibody techniques where the lysine groups of the monoclonal antibodies were joined to the alcohol groups of the dextrans.(15). Because

mab techniques are often species specific, transfection agents, such as poly-L-lysine, lipofectin, or protamine sulfate, were quickly adopted as an alternate method for internalization of U/SPIOs.

Typically, the U/SPIOs are mixed with these transfection agents, which results in complexes via electrostatic interactions of the two agents.(16,17) Care must be taken to titrate the concentration of both agents so that good cellular uptake occurs without the formation of precipitates of the complex. Using 25 μg Fe/ml ferumoxides (Feridex, Berlex Laboratories, Inc.) and 375 ng/ml p poly-L-lysine (PLL), the complex is then incubated with the cells in culture media for 24-48 hours resulting in a consistent endosomal iron uptake of 10-20 pg Fe per cell in a wide variety of cell lines without species specificity.(18-25). While Feridex-PLL labeling of human mesenchymal stem cells (MSCs) does not affect cell proliferation or viability, chondrogenic differentiation assays performed *in vitro* were inhibited whereas osteogenic and adipogenic differentiation were not impaired.(26,27) Arbab and colleagues have shown using

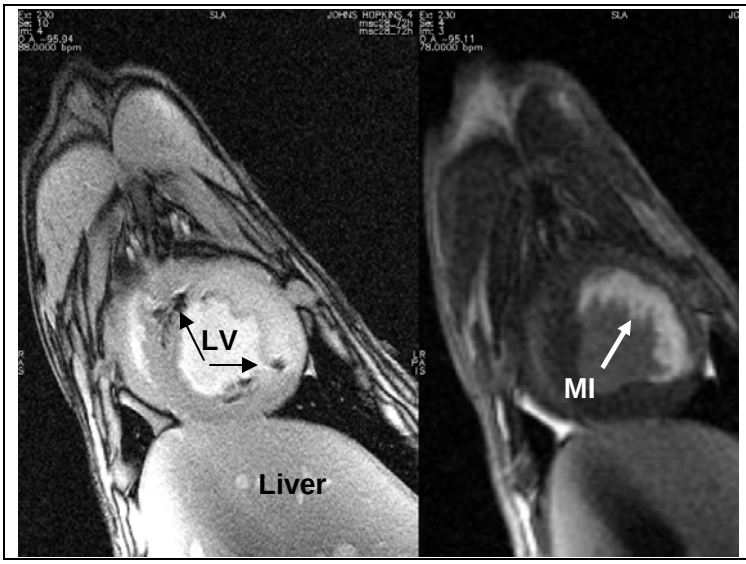

Figure 1: Short-axis images of the heart. Using a fast, gradient echo imaging technique, SPIO-labeled mesenchymal stem cells injected under x-ray fluoroscopy can be targeted to the peripheral of the infarct (left). SPIO-labeled cells appear as hypointense signal (arrows). The myocardial infarction (MI) appears hyperintense on from delayed contrast-enhanced MRI (right)

transfection of SPIOs with protamine sulfate that chondrogenic capacity may be less inhibited.(28)

More recently a method that avoids the use of transfection agents entirely has been developed called "magnetoelectroporation" or MEP.(29) MEP is based on electroporation techniques used to transfect cells with viruses or DNA. However, for MEP, a much smaller voltage pulse is applied to transfer the SPIO into the cell. No affect on cell viability, proliferation, or differentiation was noted in a variety of cells after MEP. In addition, the possibility exists to use MEP to transfer other MR contrast agents intracellularly. The primary benefit of MEP is that cells can be labeled in a few milliseconds whereas PLL-SPIO-transfection techniques required cell culturing for 1-2 days.

SPIO-labeled stem cells have been used for targeted delivery and tracking of stem cells in both acute and chronic myocardial infarction animal models.(21,22,30-38) Using MR fluoroscopic delivery of SPIO-labeled stem cells, the cells can be targeted to specific regions of the infarcted heart, and the success of each injection can be immediately ascertained.(30,31,35) Studies are currently ongoing to test the efficacy of specific stem

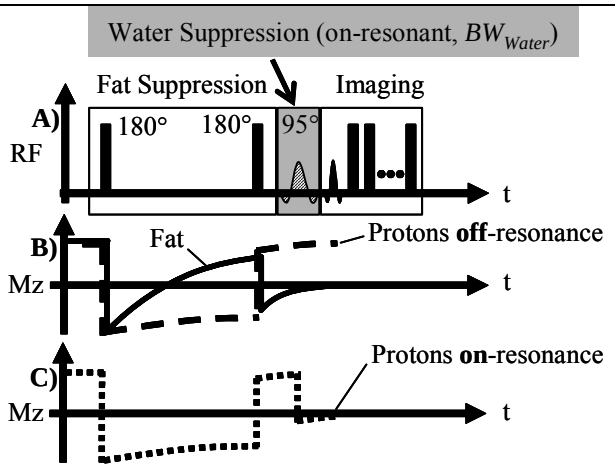

Figure 2: Positive marker imaging of SPIO-labeled cells using Inversion Recovery with ON resonant water suppression (IRON). A spectrally-selective radiofrequency pulse is used to suppression water. Fat suppression can be obtained with a variety of techniques including double inversion recovery as shown here. IRON prepulses are played out prior to the normal imaging sequence.

cells or progenitor cells for cardiac regeneration as well as determining the optimal timing of stem cell injections. However, all techniques suffer from the inability to distinguish stem cells containing SPIOs to stem cells that have died and left the iron nanoparticles behind. Moreover, the detection limit of SPIO-labeled stem cells still remains far below the detection limit of radionuclide techniques.(39)

Enhanced Methods for Imaging SPIO-labeled Cells:

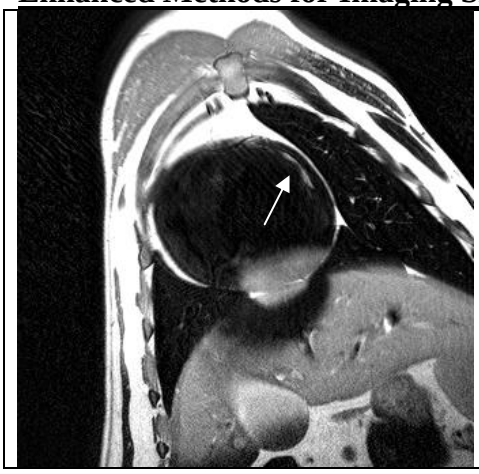

Figure 3: Inversion Recovery with ON resonant water suppression (IRON) of a short-axis image of a heart after intramyocardial injection of SPIO-labeled mesenchymal stem cells. SPIO-labeled stem cells appear hyperintense with IRON (arrow) imaging rather than hypointense as seen with T2-weighted imaging techniques. The myocardium appears dark using the IRON technique.*

Typically, SPIO-labeled stem cells exploit the signal void or susceptibility artifact created by the iron compound. (Figure 1) Recently, several groups have modified a technique developed by Seppenwolde and co-workers (40) to create positive signal from the SPIO-labeled cells. All these positive-marker techniques (41-43) rely on imaging the "off-resonant" protons created by the perturbation of the local magnetic field by the iron-labeled cell (Figure 2). One technique developed by Coristine et al. uses dephasing-rephasing gradients such that only the off-resonant protons are not rephrased and appear bright. Another technique by Cunningham et al. employs a spectrally-selectively pulse to excite a small spectrum of off-resonant protons to create a hyperintense signal close to the cells. This technique has been used to image SPIO-labeled embryonic stem cells in a

mouse hindlimb ischemia model. The final technique by Stuber et al. uses an Inversion Recovery with ON resonant water suppression (IRON) to suppresses signal from on-resonant water and fat. Theoretically, the contribution of off-resonant protons is greatest with IRON resulting in the high contrast-to-noise (CNR) ratio of the positive marker techniques. IRON MRI has been used to image SPIO-labeled stem cells in beating heart (Figure 3).

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